Comparison of Two Methods to Measure Macular Pigment Optical Density in Healthy Subjects

Catherine Creuzot-Garcher, Philippe Koehrer, Caroline Picot, Serge Aho, and Alain M. Bron

1Department of Ophthalmology, University Hospital, Dijon, France
2Department of Epidemiology, University Hospital, Dijon, France

Correspondence: Catherine Creuzot-Garcher, Service d’Ophthalmologie, CHU Dijon 1, Boulevard Jeanne d’Arc 21000 Dijon, France; catherine.creuzotgarcher@chu-dijon.fr.
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Objective. To evaluate and compare macular pigment optical density (MPOD) measurements obtained using the modified Heidelberg Retina Angiograph (HRA) and the Visucam 200.

Methods. Healthy young subjects were included in this prospective study. MPOD was measured with the modified HRA at 0° and 0.5°, 1°, 2°, and 6° eccentricities from the fovea. The parameters obtained with the Visucam 200 (maximum, mean, area, and volume) were recorded the same day on the same subjects. Intraclass correlation coefficients (ICCs) and correlation coefficients were used to evaluate the agreement between the two devices. The repeatability and the reproducibility of each method were also assessed.

Results. Sixty-seven subjects were included whose median (interquartile ratio) age was 25 years (range, 23–30 years). The MPODs as measured with the modified HRA were higher than those measured with the Visucam 200 (P < 0.0001). The ICCs were low, ranging from 0.020 to 0.188. The correlation coefficients between the two methods were very low and ranged from 0.05 to 0.22. Repeatability and reproducibility were good with both methods, with ICCs ranging from 0.697 to 0.923.

Conclusions. Agreement between the modified HRA and the Visucam in measuring MPOD was rather low. These results suggest that the two methods are not interchangeable. Before using the Visucam 200 in clinical and research setting, further evaluation seems mandatory (http://ansm.sante.fr/ number, 2009-A00418-49).

Keywords: retina, macular pigment, two-wavelength fundus autofluorescence, fundus reflectometry

Macular pigments (MPS) are mainly made up of two carotenoids: lutein (L) and zeaxanthin (Z). Located in the Henle fibers and in the inner plexiform layer, the highest MP density is found in the fovea.1,2 These fibers play an important role in protecting the retina against oxidative stress through different mechanisms.3 The amount of incoming short wavelength blue light is decreased by their absorbance spectrum, with specific absorption between 400 and 500 nm, peaking at approximately 460 nm. MPS also act as scavengers against free radicals formed by oxidative stress.

Humans cannot synthesize MPS and dietary intake—i.e., fruits, vegetables, and egg yolks—is the only source for the body. Some epidemiological studies have shown that higher levels of MPS are associated with lower risk of AMD, but the results were controversial, some studies showing a link between MP and AMD1–6 while others did not.7–9 More recently, in the AREDS 2 (Age-Related Eye Study) prospective ancillary study, it was shown that a reduction in the fovea of MPS was associated with a lower risk of AMD progression.10 Betacarotene substitution by lutein was mainly suggested because of the increased risk of lung cancer in former smokers. Several studies have assessed the possibility of increasing MPS in the retina through an increased MP dietary intake with supplementation.11,12 Finally, little is known about the potential beneficial role of MPS as a primary prevention strategy in at-risk patients.

To better evaluate the ability of MP supplementation to reach the target tissue (i.e., central retina), an objective, simple, and reliable method is needed to evaluate macular pigment optical density (MPOD) in clinical practice. Such MP measurements would allow clinicians to determine the population with low MPOD that could potentially benefit from increased MP intake. Indeed an ancillary study in AREDS patients showed that, before any supplementation, MPOD was high in some patients at baseline, making supplementation less relevant.13 Furthermore, such a device could make it possible to follow MPS in vivo during longitudinal studies. Many methods aiming to measure MPS in the retina have been described, based on either psychophysical or physical techniques.2 Among the latter, fundus autofluorescence has been proposed as a useful tool based on the fluorescent properties of lipofuscin. These devices are based on the assessment of MP distribution known to decrease from the highest level centered on the fovea with a gradual decrease toward the periphery, reaching negligible levels of MPS after 6° or 7° eccentricity with different profiles.14 The modified Heidelberg Retinal Angiograph (HRA; Heidelberg Engineering, Heidelberg, Germany) and the modified scanning laser ophthalmoscope (SLO; Heidelberg Engineering) used two-wavelength fundus autofluorescence (AF) to evaluate MPOD based on the pioneering work of Delori et al.15 More recently, another technique based on reflectometry, the Visucam 200 (Carl Zeiss Meditec AG, Jena, Germany)—measuring MPOD through reflectance of a single 460-nm wavelength—has been proposed.16
Macular Pigment Optical Density Measurement

**MPOD Visucam Measurements.** The optional macular pigment density module for the Visucam 200 used the reflectance of a single 460-nm wavelength based on a single blue-reflection fundus image to determine MPOD and its spatial distribution.\(^{16}\) A shading correction is used that approximates the reflectance of the fundus in absence of MP. It is based on a three-dimensional parabolic function automatically fitted to fundus reflectance at peripheral locations.\(^{16}\) The subject was positioned in front of the fundus camera and instructed to look at a target inside. The fundus was illuminated by a monochromatic blue light. Four MPOD parameters were automatically calculated: maximum optical density (MPOD measured at the peak); mean OD (mean MPOD within the area of macula); area (area where macular pigment could be detected); and volume (sum of all optical densities, as recommended by the manufacturer).

**MPOD Modified HRA.** Each participant was positioned in front of the SLO camera and instructed to look straight ahead. After focusing the SLO on the macular region, sequences of 20° AF images were captured at 488 nm (well absorbed) and 514 nm (minimally absorbed) at least 30 seconds after retinal bleaching.\(^{17}\) MPOD maps were generated by digital subtraction of the log AF images. We recorded MPOD at 0° and we located circles centered on the fovea at eccentricities of 0.5°, 1.0°, 2.0°, and 6.0°, and the mean MPOD values were calculated for each using the software provided by the manufacturer of the device. MPOD was expressed in DU. Typical MP distribution has a central peak or a ring-like pattern and MPOD was in most cases optically undetectable at 6° eccentricity. We excluded poor-quality maps from the analysis and analyzed the best MPOD map.

Intraexaminer repeatability was tested by the same investigator on a subgroup of 26 subjects with two successive MPOD measurements taken the same day (intrasession repeatability) and 1 month later with both methods (intersession repeatability). Interexaminer reproducibility was assessed in the same subset of participants the same day with both techniques with two different trained examiners. The examination conditions were similar, as described above (pupil dilation, sequence: Visucam and then HRA, selected eye).

### Statistical Analysis

We studied both eyes but only one eye was selected for statistical analysis: the right eye was chosen for people with an even-numbered date of birth and the left eye for an odd-numbered date of birth. Visual acuity was measured using Snellen charts and was then transformed into the logarithm of the minimal angle resolution (logMAR) units for statistical analysis. Data were reported as the median (interquartile range) since the continuous variables were not normally distributed (Kolmogorov-Smirnov test). Comparisons were made with the Fischer exact test for dichotomous data. Nonparametric tests were used for the comparison of continuous variables, the Mann-Whitney test or the Wilcoxon test for nonpaired and paired variables, respectively. The Spearman rank correlation coefficient was used for correlations. The agreement between MPOD measurements obtained with the modified HRA and the Visucam 200 were evaluated using the intraclass correlation coefficient (ICC). Since the various metrics of each method were highly correlated we only included the comparisons of MP measurement at 0 and 2° for the HRA and the max and mean values for the Visucam.

The relationship between MPOD and each of the explanatory variables (age, sex, BMI, smoking, ring-like structure) was modeled as a multilinear regression with a robust variance estimator. All analyses were conducted using statistical software (Stata version 12.0; StataCorp LP, College Station, TX, USA). The level of statistical significance was set at \(P < 0.05\) and the tests were two-tailed.

### Results

**MPOD Measurements**

MPOD measurements with each technique are displayed in Table 1. The correlation of MPOD with the Visucam 200
between the right eye and the left eye was 0.75 and 0.78 for the maximum and mean MPOD value, respectively. The coefficients were slightly lower for the modified HRA, 0.66 and 0.68 at 0° and 2° eccentricity, respectively. For comparisons between the two methods, we retained only one eye per subject, as mentioned above. Since the two devices did not assess the same area, we made the following assumptions: MPOD measured with the modified HRA at 0° eccentricity could be considered as close to maximum MPOD measured by the Visucam 200. Similarly, MPOD measured with the modified HRA within a 2° eccentricity could be considered as close to the mean MPOD measured by the Visucam 200.

We found a parafoveal MP ring pattern with the modified HRA in 41 (61%) of the participants. However, it was difficult to see such a ring with the Visucam 200 and this type of classification with this technique does not seem reliable. In fact, the Visucam 200 displays a sort of “en-face image” and a 3D representation with a small scale. Conversely, the HRA layout is a section with a high magnification, which provides a more reliable description of the MP pattern.

The MPOD values at 0° and within 0.5°, 1°, and 2° eccentricity obtained with the modified HRA were significantly higher than the maximal MPOD values obtained with the Visucam 200 (P < 0.0001, Wilcoxon signed-ranked test). But the MPOD values at 6° eccentricity obtained with the modified HRA were lower than the mean MPOD obtained with the Visucam 200 (P < 0.0001, Wilcoxon signed-ranked test).

The correlation coefficients comparing MPOD measured with the two machines were low, ranging from 0.05 to 0.22 (Fig.). The ICCs were low as well, ranging from 0.010 to 0.104.

In multivariate analysis, taking MPOD at 0° eccentricity with the modified HRA and the maximum value with the Visucam 200 as a dependent variable and age, sex, BMI, smoking and a ring-like MP pattern as independent variables, we did not identify any statistically significant influence of these independent variables on MPOD measurements for either method.

Reproducibility
Table 2 shows intraexaminer repeatability and interexaminer reproducibility for MPOD measurements with the two devices in the subgroup of 26 young healthy subjects. Overall, the variability between measurements was low with the Visucam 200 and the modified HRA. The range of variations for both instruments was acceptable from a clinical perspective.

Discussion
There are many arguments in favor of considering MP as playing a protective role against AMD based on the antioxidative properties of carotenoids.18 Many factors are known to influence MPOD: age, obesity, smoking, and genes involved in the transportation of lutein along the lipid pathway.19 Surprisingly, the influence of modifying dietary carotenoid intake on final MPOD seems to be highly variable.20

Studies that assessed MPOD are based on two main methods, both with advantages and drawbacks. The detailed review made by Howells et al.2 clearly demonstrated that to date there has been no clear gold standard technique to measure MPOD. Clinicians have to take into account several parameters before selecting the most appropriate technique for their population: the age of the patients and their lens status, their ability to respond to psychophysical testing, the cost of the machine, fixation ability, etc. All these factors can explain the controversial results found in the literature: some studies showed a decrease of MPOD with age21; others found no age dependency22 while another reported an increase of MPOD with age.23 The relation of carotenoids and other nutritional factors such as omega-3 fatty acids among non-modifiable genetic risk factors make drawing conclusions even more difficult.24

The modified HRA used two-wavelength (488- and 514-nm) fundus AE15,25–28 based on the AF of lipofuscin, which is normally located in RPE cells.29 To measure the MPOD, the dual-wavelength method compares results from excitation wavelengths that are differentially absorbed by the MPs, thereby taking into account the nonuniform distribution of lipofuscin in the RPE.28 Several studies have found MPOD variability among different techniques and patients.17,21,25,30,31 Lens opacity is a confounding factor, and since it was shown that cataract decreases MPOD measurement, only subjects with clear crystalline lenses were recruited in the present study.32 The one-wavelength reflection method is a simplified method for measuring OD. Fewer studies have used reflectance of a single 460-nm wavelength.16 Moreover, we do not know from the manufacturer which area is actually measured. It seems that this could change from one patient to another. Our MPOD values measured with the modified HRA were consistent with those found in the literature. In a normal population, some studies reported comparable MPOD values.
TABLE 2. Intraexaminer Repeatability (Intra- and Interession) and Interr Examiner Reproducibility of MPOD Using the Modified HRA and the Visucam 200

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<tr>
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<th>Intraexaminer Repeatability</th>
<th>Interexaminer Reproducibility</th>
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<tr>
<td></td>
<td>Intrasession</td>
<td>Limits of Agreement</td>
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<tr>
<td>Modified HRA</td>
<td></td>
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<tr>
<td>MPOD within 0°</td>
<td>0.923 (0.837, 0.965)</td>
<td>-0.159 to 0.103</td>
</tr>
<tr>
<td>MPOD within 2°</td>
<td>0.824 (0.646, 0.917)</td>
<td>-0.152 to 0.101</td>
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<tr>
<td>Visucam 200</td>
<td></td>
<td></td>
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<tr>
<td>Mean</td>
<td>0.842 (0.689, 0.929)</td>
<td>-0.018 to 0.022</td>
</tr>
<tr>
<td>Maximum</td>
<td>0.916 (0.821, 0.961)</td>
<td>-0.034 to 0.039</td>
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CI, confidence interval.

for the modified HRA: 0.44 ± 0.11 DU for 0.5° eccentricity and 0.11 ± 0.05 DU for 1.75° eccentricity (n = 17; age range, 30 years); 0.51 ± 0.12 DU at 0.5° eccentricity; 0.29 ± 0.07 DU at 2° eccentricity (n = 14; age range, 56 years); 0.50 ± 0.20 DU at 0.5° eccentricity (n = 136; age range, 71 years); and 0.24 ± 0.10 DU at 2° eccentricity (n = 146; mean age, 71 years). On the contrary, one of the few studies analyzing MPOD with the reflectance method by Visucam 200 has shown higher maximal MPOD values than what we have found in the present study: 0.62 ± 0.10 DU in pseudophakic eyes (n = 22) and 0.67 ± 0.10 DU in phakic eyes (n = 47) for maximum MPOD (mean age, 72 years). In a more recent interventional study using the same technique, the baseline measurements before carotenoid supplementation were not provided. 11,16

This study aimed to compare MPOD measurement in healthy patients without blurred media or retinal diseases. We did not find good agreement between MPOD values measured by modified HRA and Visucam 200. The MPOD measurement was reproducible for each device, but the data collected by the two techniques could not be compared. Agreement between the modified HRA and the Visucam 200 has been studied only once in a study involving patients suffering from AMD. 16,17 Schweitzer et al. 16 found a good correlation (r² = 0.855) in their study population (n = 19; age range, 60–87 years). However, the values used in their analysis from the Visucam 200 were defined as the values found in “an annulus with a radius of 0.5°,” which is not available in the commercialized device. 16

We found good repeatability and reproducibility of MPOD measurements with both instruments. Macalett et al. found good reproducibility of the Visucam MPOD module: the coefficient of variation of three measurements by one examiner was 1.07% ± 3.1% for maximum MPOD and 1.8% ± 3.1% for mean MPOD (Machalett et al., unpublished observations, 2011; A new reflectance method to determine the macular pigment: investigation of reproducibility and variability). Schweitzer et al. 16 reported reproducibility of all parameters at ~6% and similar results were found by others. 14,15,19,54 Several studies showed better reproducibility for the AF method than our study, but these were often intrasession evaluations. 57,20,34,36

What could motivate clinicians and researchers to choose one technique over another? The dual-wavelength AF method has been the most thoroughly studied and is one of the most widely used physical methods. Several studies have shown good agreement between the “modified HRA” technique and nonphysical techniques such as heterochromatic flicker photometry 57 and motion photometry. 58 However, examinations require pupil dilation, a bright light and manual operation to identify the center the fovea in a certain number of cases. In its classical version, the Heidelberg device is expensive and requires the customization of an old HRA, which very often is not available. Moreover, it is only dedicated to MP measurement. Very recently the module for measuring MP was integrated into the Heidelberg Spectralis HRA-OCT Multicolor (Heidelberg Engineering GmbH, Heidelberg, Germany), but few studies have evaluated this new device. 37 The Visucam 200 (Carl Zeiss Meditec AG) was released on the market a couple of years ago in some countries and combines a nonmydriatic camera with an MP measurement module based on reflectometry. It is easy to use with fully automated acquisition in only one operation and quite reproducible, but few studies have been published on this device to date. 11,16,37,39 The MP measurements, as was previously described for reflectometry, 15 are lower with the Visucam 200 than those recorded with other techniques. This has been confirmed in two recent papers. 37,39 Therefore we may wonder whether the Visucam 200 based on reflectometry is really measuring MPOD or something else which is not yet clearly identified.

Various limitations to the present study should be acknowledged: we did not perform any serum carotenoid measurements to assess the relation between serum carotenoids and MP measurement. However, the goal was not to evaluate the relation between MPOD and serum carotenoids, but rather to compare two MPOD measurement methods. This study included a small population but the MPOD measurement conditions were optimal, with young cooperating subjects without any interfering disease leading to MPOD measurement bias. This is certainly also a weakness since in an actual clinical population with older patients and anterior and posterior segment diseases, MPOD measurements could well be more difficult to take and less reliable.

The findings reported herein show that although reproducibility was good for both methods, the agreement between MPOD measured by the modified HRA and the Visucam 200 was poor. To date few studies have analyzed MPOD values through fundus reflectance with the Visucam 200. These results suggest that these two methods are not interchangeable. It is therefore too early to recommend the Visucam 200 for MP determination in clinical research or in a routine practice and further evaluation is needed to better clarify what the Visucam is really measuring.

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